Structure-Function Analysis of Lamina-Associated Polypeptide 1 and its Role in the Activation of the AAA+ ATPase, TorsinA

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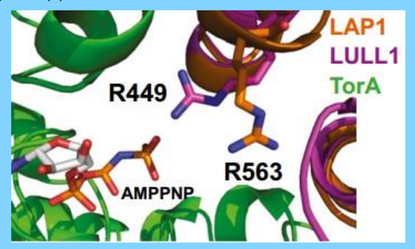
Abstract

Torsion dystonia is a neurological disease caused by a mutation in *DYT1*. This gene encodes the AAA+ ATPase, TorsinA (TorA), which is involved in regulating nuclear movement in polarizing fibroblasts. The mechanism by which this mutation causes torsion dystonia is not understood. The inner nuclear envelope protein Lamina-Associated Polypeptide 1 (LAP1) is required to activate TorA ATPase activity. The crystal structure of the LAP1 luminal domain suggests that arginine 563 is necessary to activate TorA. It also suggests that cysteine 424 and 582 form an intramolecular disulfide bond that may be important for the LAP1 luminal domain to adapt the correct structure. In addition, cysteine 496 is highly conserved and may play a role in TorA activation. TorA knockout fibroblasts exhibit defects in nuclear positioning. Using the wounded fibroblast monolayer system we found that siRNA knockdown of LAP1 inhibited nuclear positioning in polarizing fibroblasts. This nuclear positioning defect was rescued by microinjection of cDNA constructs encoding GFP-LAP1^{WT} and GFP-LAP1 with either R563E, R563A, or C496S mutations. The nuclear positioning defect was not rescued by microinjection of cDNA construct encoding GFP-LAP1 with either C424S or C582 mutations.

Introduction

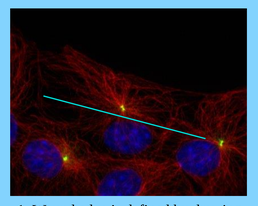
- Early-onset torsion dystonia is a neurological disorder caused by a mutation deleting a single glutamate residue in the AAA+ ATPase, TorsinA (1), however the disease etiology is poorly understood.
- TorA regulates nuclear movement and positioning through interaction with the LINC complex, a nuclear envelope complex required for nuclear movement in polarizing fibroblasts (2).
- TorA is activated by Lamina-Associated Polypeptide 1 (LAP1), an inner nuclear membrane protein (3).
- Crystal structure of the LAP1 luminal domain suggests R563 required for TorA stimulation (4).
- Structural data also suggests C424 and C582 form an intramolecular disulfide bond that may be important for LAP1 function (4).
- C496 is highly conserved and may be important in TorA stimulation (4)
- To test these predictions, we created GFP-tagged LAP1 constructs with mutations in these residues.
- Tested ability of constructs to rescue nuclear positioning in cells treated with LAP1 siRNA using the wounded fibroblast monolayer system (5).



Zoomed in view of the crystal structure of LAP1 TorA interface, showing LAP1 R563 in close proximity to the TorA ATP binding site. (6)

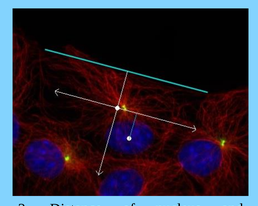
Methods & Materials

- GFP tagged LAP1 constructs were created: wild type (WT), R563E, R563A, C496S, C424S, and C582S
- NIH3T3 fibroblasts were treated with LAP1 siRNA and allowed to grow on glass coverslips for 48 hours.
- Fibroblasts were serum-starved for 24 hours to induce quiescence.
- Artificial wounds were created and wound edge cells microinjected with LAP1 constructs.
- Fibroblasts were stimulated with the serum component, lysophosphatidic acid (LPA), which induces polarization toward the wound edge.
- Cells were fixed after two hours of stimulation and visualized using immunofluorescence.
- Using custom MATLab code, nuclear and centrosome positioning and orientation of the nuclear-centrosomal axis toward the wound edge were measured as follows.

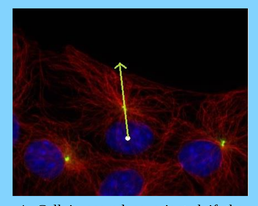


1. Wound edge is defined by drawing line between contact points with two adjacent cells .

2. Cell border is traced and centrosomes are defined.



3. Distance of nucleus and centrosome from cell centroid along axis perpendicular to the wound edge is measured.



4. Cell is scored as oriented if the nuclear-centrosomal axis passes through the wound edge.

Results

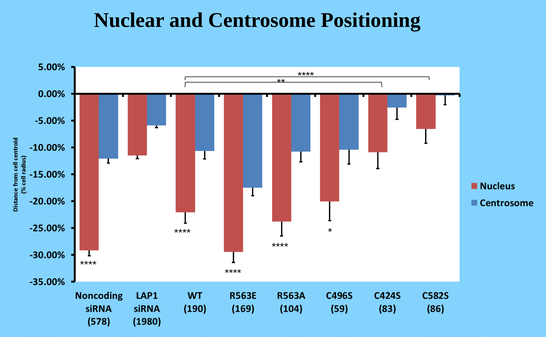


Figure 1. Nuclear and Centrosome Positioning in Rescue Experiments. NIH3T3 fibroblasts were treated with siRNA against LAP1. Cells at the wound edge were starved, injected with the indicated GFP LAP1 construct, stimulated with LPA, and the nuclear and centrosome positions were measured and compared to cells treated with non-coding siRNA. The cell center corresponds to 0 on the y-axis. Numbers in parentheses represent number of measured cells for each treatment. Asterisks below bars show significance between treatment and LAP1 knockdown nuclear positioning.
* = p< 0.05, ** = p < 0.01, *** = p <0.001, **** = p < 0.0001

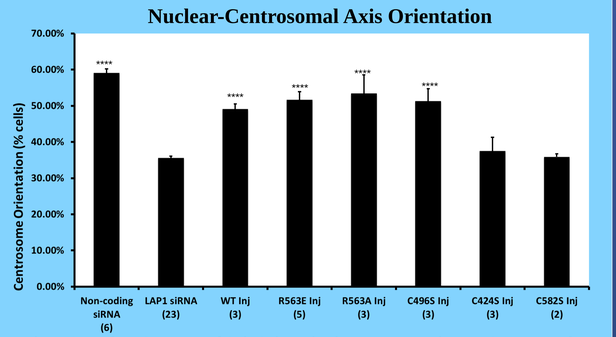


Figure 2. Nuclear-Centrosomal Axis Orientation in Rescue Experiments. Orientation of the nuclear-centrosomal axis was scored in uninjected and injected LAP1 knockdown fibroblasts at the wound edge. Numbers in parentheses represent number of replicates. Asterisks above bars show significance between treatment and LAP1 knockdown centrosome orientation. * = p< 0.05, ** = p < 0.01, *** = p <0.001, **** = p < 0.0001

- Treatment of fibroblasts with LAP1 siRNA inhibited nuclear positioning and decreased orientation of wound edge cells compared to cells treated with non-coding control siRNA.
- GFP-LAP1^{WT} rescued nuclear positioning and nuclear-centrosomal axis orientation in cells treated with LAP1 siRNA.
- The GFP-LAP1^{R563E} mutant rescued nuclear positioning and orientation more strongly than GFP-LAP1^{WT}, suggesting R563E may be a gain of function mutation.
- The GFP LAP1^{R563A} and GFP LAP1^{C496S} mutants rescued nuclear positioning and orientation to WT levels, suggesting that both amino acids are dispensable for LAP1 function.
- The GFP LAP1^{C424S} and GFP LAP1^{C582S} mutants did not rescue nuclear positioning or orientation, suggesting that this intramolecular disulfide bond in the LAP1 luminal domain is important for nuclear positioning.

Future Directions

- Since GFP-LAP1^{R563E} showed greater rearward nuclear positioning than GFP-LAP1^{WT} incells treated with LAP1 siRNA, we may want to test the hypothesis that this is a gain of function mutation by overexpressing this construct in both unstimulated and stimulated 3T3s to test whether nuclear positioning and orientation are enhanced.
- Since the both C424 and C582 were important for LAP1 function, we want to make the double mutant construct (GFP-LAP1$^{C424S/C582S}$) to test whether inhibition of nuclear positioning and orientation is worsened.
- Generate GFP-LAP1 luminal domain and nucleoplasmic domain cDNA constructs to test whether either domain alone is sufficient to rescue nuclear positioning and orientation.
- Generate GFP-LAP1 constructs with point mutants at other conserved arginine residues and perform rescue experiments to study whether other arginine residues are important for LAP1 function.

References

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